## 2017 National Rice R&D Highlights

# HYBRID RICE PROGRAM





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### Hybrid Rice Program

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#### **Executive Summary**

Using "heterosis" or hybrid vigor in rice cultivation, which result in at least 15% yield advantage over inbred, is a viable strategy of increasing rice production and productivity per unit paddy area. To increase rice production in the country, the Department of Agriculture targets to cultivate 1M hectare of farms with hybrid by 2019 with 25% to be planted with public hybrids. This challenge entails development of hybrid varieties and parents, which are adaptive, adoptable, and with commercialization potential across diverse target production environments, as well as developing associated crop management technologies that enable optimal expression of the yielding ability of the hybrids and parents.

The Hybrid Rice Program focused primarily in rice breeding and breeding research to develop high performing, good quality, and insect pest and disease resistant CMS (cytoplasmic male sterile)-three line and TGMS (thermo-sensitive genic male sterile)-two line hybrids that would increase grain yield and income of farmers. It is also engaged in seed production research to increase seed yield, reduce the cost of seed, and hasten the adoption and dissemination of the hybrid rice technology. Moreover, the program addressed crop management research to increase grain yield and reduce cost of hybrid rice production. It also included research on seed purification and multiplication techniques for nucleus and breeder seed production and maintenance, seed quality testing, improvement of seed quality standards, and technical support in hybrid seed production and seed certification training.

The Program aimed to develop wide-adaptive, high-yielding hybrid rice varieties with good agro-morphological traits, acceptable grain and eating quality traits, and resistance to major pests. It also ensured sustained adequate supply of genetically pure and high quality nucleus and breeder seeds of parents and F1 hybrids in support to hybrid rice commercialization.

The Program explored development of cost-reducing technologies for specific growing environments such as mechanized and direct seeding crop establishment, as well as expand target production environment to favorable rainfed areas for hybrid cultivation as viable sources of growth in rice production. The Program target outputs included, among others, quality seeds of parents and hybrids, F1 seed production methodologies, and crop and seed management technologies.

### **I. Development of CMS-Based Three-Line Hybrids** Joanne D. Caguiat

CMS is the first male sterility mechanism discovered and is widely used in hybrid development. A three-line system comprises of a male sterile line (CMS- or A-line), a maintainer line (B-line), and a fertility restorer line (R-line) (Islam et al., 2015). Maintainer and restorer lines are very essential components in the development of hybrid rice and in the multiplication of CMS lines and F1 seed production. Continuous research on the identification of inbred cultivars that can either maintain the sterility or restore the fertility of CMS lines is necessary in developing high-yielding germplasm pools. Parent lines are diversified through the development of new germplasm and improvement of existing CMS, maintainer, and restorer lines.

The project comprised ten studies primarily focused on the development of high yielding, pest and disease resistant parent lines (maintainer and male-sterile lines), and superior experimental hybrids. Parent line development includes studies on phenotypic and genotypic characterization, generation of useful variation through mutagenesis, and use of wide-compatibility genes for enhancing heterosis of parent lines and hybrids. Studies also involved the performance test evaluation, yield prediction, and seed production of experimental hybrids. The project also engaged on increasing seed yield of parent lines and hybrids based on outcrossing rate, row ratio and leaf number counting for synchronization. These studies were interrelated to attain the project objectives: a) develop new and diverse hybrid parent lines, male sterile lines (A- and S- lines), and pollen parents (R- and P- lines); b) improve the morpho-agronomic characteristics of parent lines; c) develop, evaluate and identify superior three-line hybrids; d) produce high purity and high quality seeds of experimental hybrids; and e) conduct multi-location yield trials of parentals and promising hybrids.

For parent line development, the project developed and selected 20 potential restorer lines with 8 t/ha yields and 80% fertility, three advanced maintainer lines with 7.2-7. 6 t/ha yield, maintainer lines with 87-100 % stigma exertion rate for increase F1 seed yield, and a new potential CMS line (PR46622A) with complete sterility and good flowering behavior. Moreover, 64 potential restorer and 53 potential maintainer lines were prospected and forwarded to the restorer and maintainer line development.

Study on mining of wide-compatibility genes to resolve fertility barrier and to facilitate possible transfer of novel gene from other cultivar to modern varieties was also conducted. Using S5-MMS marker, one inbred line exhibited positive for S5n allele and showed >70% fertility when crossed to both Indica and Japonica tester. Among Javanica germplasm screened, 13 lines also showed positive for S5n allele. Further evaluation will be done to confirm the presence of the S5n allele.

Screening of hybrid parent lines and hybrids for biotic stress resistance was done to identify resistant lines to be used in varietal development and improvement. Eighty-one lines have resistance to blast, 24 BPH-resistant, 32 GLH-resistant, 34 tungro-resistant, and 60 BLB-resistant. Molecular analysis was conducted using markers for Xa, Glh14, and tsv1. Glh14 and Xa4 (which confers low resistance to BLB) were found in most of the lines. Identified resistant lines will be utilized in hybrid and parent line development.

Evaluation of experimental hybrids in observational nursery (ON), preliminary yield trial (PYT), and multi-location yield trial (MYT) were conducted to assess the performance of hybrids in terms of yield, resistance to pest and diseases, and other morpho-agronomic traits. Twelve hybrids in ON; three, PYT; and two in MYT were selected based on its  $\geq$  5% yield advantage over the hybrid and inbred check varieties. These hybrids will be seed produced and advanced to corresponding nurseries for further evaluation. Meanwhile, the demonstration trial of released hybrids in Isabela showed yield of Mestiso 20 with 10.02 t/ha, Mestiso 73 with 9.12 t/ha and Mestizo 1 with 6.65 t/ha.

The project also seed produced the nucleus, breeder, and foundation seeds of parent lines and experimental and elite hybrids. Foundation seed production of parent lines attained 0.20-1.8 t/ha seed yield, F1 seed production in MYT produced 0.20-1.5 t/ha seed yield; NCT hybrids seed production registered 0.6-1.5 t/ha seed yield. Only hybrids confirmed with  $\geq$ 1.5 t/ha seed yield were forwarded to advance nurseries.

Yield prediction based on best linear unbiased prediction (BLUP) was employed to predict non-observed performance of hybrids; lessening the time, effort, and labor in the development of most suitable genotypes. Correlation between actual and predicted values for effects and yield gained a high value of 91.67% and 95.68% for yield ranking. Results suggested that BLUP can be used in predicting the performance of untested hybrids and will be useful in modern hybrid rice breeding programs.

### **Development of Hybrid Parent Lines**

RA Millas, FP Waing, LV Gramaje, VP Luciano, MSF Ablaza, MM Rosario, and PLH Duran

The continuous success of hybrid rice breeding lies mainly on the development of the hybrid parent lines. Therefore, there is a need to develop new and improve existing maintainer and restorer lines. The study aimed to: (1) develop new and diverse hybrid parent lines, male

sterile lines (A-line), and pollen parents (R-line); (2) improve agronomic and morphological characteristics of parent lines; and (3) convert potential B lines into new A lines and utilize potential R lines to further enhance the restorer line genepool of PhilRice hybrid breeding program.

There were 1,060 lines in pedigree method and 967 F3-F6 lines in GWS were selected based on good phenotypic acceptability in restorer line development. In the observation yield trial, 18 potential restorer lines have 90-98% pollen fertility. Thirty-seven of 97 entries from genome-wide selection nursery out-yielded the best check. From these entries, 20 potential restorer lines with 8 t/ha yield potential and 80% fertility were identified and nominated in the hybrid source nursery.

In maintainer line development, 51 new BxB combinations for DS and 33 from WS were assembled. F1 seeds were generated and 1,620 lines in the segregating population were selected for further evaluation in DS 2018. Out of the 42 prospective maintainer lines (F6 to F8 generation) forwarded, six test entries showed remarkable yield advantage of 6-36% over the best check NSIC Rc222. Performance of these lines will be confirmed the following season.

A promising CMS line denoted as PR46622A with 100% complete sterility was identified. This will serve as a new potential CMS line with desirable traits and sterility stability that could be used in testcrossing activities and hybrid development.

### **Development of Three-line Experimental Hybrids**

VP Luciano, LV Gramaje, FP Waing, RA Millas, MSF Ablaza, PLH Duran, and JD Caguiat

Utilization of diverse germplasm continuously plays a significant role in hybrid rice breeding especially in increasing heterosis. Capitalizing on diverse parental lines with better agronomic traits is proven to be an effective strategy in developing superior three-line hybrids. Therefore, generation of experimental hybrids from the different breeding pool by testcrossing is imperative. This study aimed to generate experimental hybrids and evaluate their agronomic and morphological traits, and yield performance. Testcross from assembled 464 and 160 male parents crossed to five CMS for each season generated 1,473 and 484 experimental hybrids in DS and WS, respectively.

For field evaluation, 41 lines out of established 938 in DS showed 15.31-263.65% yield advantage over the four check varieties (Mestiso 19, Mestiso 20, PSB Rc222, and PSB Rc82). IR58025Ax TCN-694 obtained the highest yield advantage of 118.07-263.65% over the checks. Meanwhile,

PR15A x PR40496-55-2-1-4 and PR19A x PR39500-8-5-49 attained the highest yield advantage of 4.71-29.43% over the four check varieties in WS. Prospective 41 restorers and 27 maintainer lines in DS and 23 restorers and 26 maintainer lines in WS were selected based on yield, fertility, sterility, and phenotypic acceptability. Days to maturity and reaction to pest and diseases were also considered. These potential restorer lines will be forwarded for line improvement, seed production for observational nursery, and performance test while prospective maintainer lines will be forwarded for line improvement and CMS conversion through backcrossing in the succeeding season.

#### **Performance Tests of Experimental Hybrids**

JOS Enriquez, MSF Abaza, MM Rosario, LV Gramaje, and JD Caguiat

The evaluation of experimental hybrids is an essential segment of variety development. A series of field tests in ON, PYT, and MYT were conducted to assess the performance of different hybrid combinations under development. For the ON, 17 entries were evaluated in the DS and 43 entries in the WS. In 2017 DS, 12 hybrid entries obtained at least 8 t/ha and out-yielded all the hybrid and inbred checks by at least 5%. However, no hybrids achieved the same harvest for 2017 WS. In the PYT, three (PR39334H, PR49510H, and PR47779H) entries were evaluated using the same criteria.

For the MYT, 10 entries were evaluated in DS and 13 in WS at PhilRice CES, Bicol, Negros, Agusan, and SPAMAST. Only two hybrids have ≥5% yield advantage over all the checks: PR46838H (6.73 t/ha) and PR47781H (6.39 t/ha) across locations. For 2017 WS, hybrid entries were evaluated in PhilRice CES, Isabela, Bicol, Negros, Agusan, WESVIARC, CENVIARC, and SPAMAST. Only results for the trials at PhilRice CES and Isabela are available and analyses will resume after the results from other sites are gathered.

#### Seed Production of Experimental Hybrids

LV Gramaje, FP Waing, MSF Ablaza, and JD Caguiat

In hybrid seed production of three-line hybrids, maintenance and purification should be given priority to the three parents: CMS (A), maintainer (B), and restorer lines (R). Nucleus seeds play an important role to the breeders because they serve as core seeds. To maintain the genetic purity, identified cross combination of A and B parents should always be in pairs as they reproduce every season regardless of seed classification (nucleus, breeder, foundation, and registered). Nucleus seeds should be produced first for A, B, and R parents. Paired crossing was done for CMS and maintainer to obtain the core seeds followed by CMS multiplication to produce the breeder seeds. Breeder seeds of R-line were purified using panicle to a row method. In DS 2018, foundation seeds were purified using the same method for the select hybrid parents. For hybrid entries requiring bulk amount of seeds for its multi-location trial, foundation seed production are established in larger plots. Seed production of parents and nominated experimental hybrids were simultaneously done provided that the hybrid entries from ON were selected and advanced to the succeeding trials. Seed production is considered a very crucial component of hybrid technology because its commercialization necessitates the availability of seeds for largescale cultivation. The seed production process for hybrid parents and F1 should be precise to ensure high quality in terms of physical and genetic purity. Thus, important traits of A, B, and R lines of each hybrid should be evaluated, monitored, and subjected to in-house and external seed quality control.

CMS multiplication of two experimental hybrid parents was accomplished. IR79128A yielded 8,110 g while IR73328A produced 2,438 g, which was obtained by selecting only the completely sterile plants in the plot through microscope evaluation. The produced is intended for breeder seed production of Mestiso 55 in the next cropping season.

F1 seed production for the observational nursery (SPON), preliminary yield trial (SPPYT), multi-location yield trial (SPMYT), and the national cooperative tests for hybrids (SPNCT) were done. Forty-one cross combinations for SPON with at least 300 seeds per combination were seed produced. Through AxR row-crossing, 10 experimental hybrids were produced for 2017 WS PYT evaluation. Following the row-crossing method, 17 experimental hybrids for MYT were seed produced with accumulated seed yield of 6035 g. The seed yield ranged from 0.20 t/ha to 1.5 t/ha. To augment current seed stock, F1 seeds of eight promising hybrids under testing in the NCT-HYB were produced in the F1 SPNCT with a total weight of 22.28 kg. Meanwhile, the seed yield for NCT-HYB ranged from 0.6 t/ha to 1.5 t/ha. To complete the seed requirements for NCT, five experimental hybrids: PR47216H, PR46951H, PR47795H, PR46955H, and PR40640H with seed yield range from 460 g to 3,335 g were produced.

Maintenance and ensuring the availability of seeds of released hybrids (Mestiso 48 and Mestiso 55) for pre-commercialization was also included in the study. Nucleus seed produced for IR79128A (M55 parent) was 11.44 kg from 44 plots while 12.10 kg of IR79128A in 32 plots was obtained from IR73328A (M48 parents). For basic/breeder seed, IR79128A obtained 15.28 kg and 5 kg purified B-lines. IR79128A was produced to supply the needed requirement for A x R seed production of Mestiso 55 to support its pre-commercialization and use seed parent of upcoming hybrids. 7

During wet season, parent lines of Mestiso 55 (IR79128A x IR79128B) were used in A x B row-ratio of 2:8:2, 2:10:2, 2:12:2, and 2:14:2. Result showed that across row-ratio, no significant differences were observed for this combination. However, the row-ratio of 2:8:2 recorded the highest average seed yield of 913.33 kg/ha among row ratios. For the AxR row-ratio of Mestiso 55 (IR79128A x PR31559-AR32-4-3-2R), although no significant differences were observed, row-ratio 3:6:3 and 3:15:3 got the highest mean seed yield of 973.33 kg/ha and 955.17 kg/ha, respectively. In the wet season, 3:12:3 row-ratio recorded the highest average seed yield of 774 kg/ha, followed by 3:9:3, 3:15:3, and 3:18:3 with average seed yield of 617 kg/ha, 590 kg/ha, and 508 kg/ha, respectively.

## Generating Useful Variation in Hybrid Parent Lines through Induced Mutagenesis

MM Rosario, JD Caguiat, MSF Ablaza, and PL Duran

Utilizing induced mutation, in-vitro culture, and in-vitro mutagenesis have been proven effective in developing superior hybrid parent lines. To increase the chance of finding desired mutation, seeds of hybrid parent lines were subjected to mutation via chemical (EMS/sodium azide) and physical means (gamma irradiation). Five kilograms of seeds were sent to the Philippine Nuclear Research Institute for gamma-ray irradiation. Treated seeds (M0) were sown and grown into plants (M1) at PhilRice-CES field without selection. Seeds (M2) were harvested per surviving mutant plant and subjected to abiotic and biotic stress screenings. Forty mutant progeny lines were selected out of six putative lines (M1) and were evaluated. Assembled 85 new cross combinations 30 RxR and 55 BxB (35 DS & 20 WS) were subjected to in-vitro culture (IVC). Of these, 19 (63%) from RxR and 12 (34%) BxB exhibited callus formation. Among the 19 RxR, only 6 generated 18 DHLs while 1 BxB generated 6 plants but turned albino. Double haploid (DH) plants will be multiplied to produce enough seeds that will be used for screening on biotic and abiotic stresses and testcrossing the lines' restoring ability. The seeds will also be used in the Source Nursery (SN). Regenerants from BxB will be forwarded to maintainer line nursery to further evaluate and validate its maintaining capability when crossed with CMS lines of different cytosterility source. Ten M5 putative mutants were derived from Matatag 11 and IRGC 5999 (Pankhari 203) were assembled in SN and testcrossed to five tester lines: IR58025A, PR19A, PR21A, PR28A, and PR29A. Fifty F1 hybrids were generated and evaluated in the Testcross Nursery along with the check entries.

### **Phenotypic and Genotypic Characterization of Parent Lines and Hybrids** *FP Waing, JC Santiago, JOS Enriquez, JD Caguiat, and JM Manangkil*

Hybrid rice can produce yield that is 20% higher than inbred semidwarf varieties. However, hybrid rice production is confronted with multiple stresses posing severe yield penalties. Damage by major diseases can cause reduction in yield should plants be infected at early crop growth stages. Improving the genetic compositions of hybrid parent lines and breeding stress-resistant hybrids through molecular markers is an effective strategy to sustain rice productivity.

This study aimed to: 1) characterize hybrid parent lines and hybrids for biotic stress resistance, 2) detect the presence of known resistance genes using molecular markers, and 3) identify resistant lines to be utilized in varietal development and improvement. Ninety-six test entries comprised of promising hybrid parent lines and F1 hybrids were subjected to blast, tungro, BLB, BPH, and GLH resistance screening using established protocols under screenhouse condition. Eighty-one lines are resistant to leaf blast; 24, BPH; 32, GLH; 34, tungro; and 60, BLB. Molecular analysis was conducted using markers for Xa, Glh14, and tsv1. Glh14 and Xa4, which showed low resistance to BLB, were found in most of lines. R-42 and B-6, which were found resistant to both PXO races, were positive for both Xa4 and Xa21 genes.

Presence of the resistance genes and putative QTL were detected but did not completely translate to the observed response of some of the genotypes to the different stresses. Thus, there is need to study the molecular basis of stress resistance. Results suggest a need for further genetic improvement in developing hybrid varieties with durable stress resistance.

# Yield Prediction of Single Cross Hybrids and Combining Ability Analysis of Parent Lines

LV Gramaje, JD Caguiat, RA Millas, and JOE Enriquez

Parent line is considered as the backbone in developing hybrids because the performance of F1 depends on the quality of its parents. Traditionally, field trials or progeny tests are used to choose best parents, which include generating hundreds of crosses under field evaluation. To lessen time, effort, and labor in the development of most suitable genotypes, the use of available genetic parameter estimation, which allows the prediction of non-observed performance of hybrids should be employed. Using eleven restorer lines and three CMS lines, 33 single-cross hybrids were generated. Field performance of the generated hybrids was evaluated in the field and data on yield and important agronomic and morphological traits were collected. Yield prediction based on the performance of parents and crosses guided by the genotype data and combining ability estimates was conducted.

Hybrid performance was predicted based on predicted performance of untested hybrids, covariance between untested and tested hybrids; relatedness, Variance of General Combining Ability (VGCA), Variance of Specific Combining Ability (VSCA), phenotypic covariance among hybrids; and performance of tested hybrids or SCA. Predicted values were crossvalidated. Two sets of predicted values were used for cross validation with 9 (2 untested hybrids) and 15 hybrids (3 untested hybrids) for the first and second set, respectively. For the first group, deviation of the predicted yield ranking from the actual values has an average of 1.56. The Pearson's correlation between actual and predicted performance for effects and yield obtained 82.45% correlation and 81.66% for yield ranking. Results for the second group showed an average of value of 1 for deviation in terms of ranking. Correlation between actual and predicted values for effects and yield gained a high value of 91.67% and 95.68% for yield ranking. New sets of hybrid crosses were also generated for 2018 evaluations and validation.

# Genetic Improvement of Maintainer Lines for Increased Seed Yield and BLB Resistance with Good Grain Quality

IG Pacada, CC Ringor, MTF Celestino, MB Dela Cruz , CM Sacdalan, and TMM Pascual

Development of maintainer lines resistant to diseases is important as this may contribute for yield stability of F1 hybrids in the future. Breeding of maintainer lines with exerted stigma is a primary step to develop a seed parent or CMS line that may contribute in increasing F1 seed yield; thus, this study.

For the improved maintainer lines, PR41327B exhibited Xa4 and Xa7 genes. The validation for other existence of Xa genes and validation thru phenotyping (inoculation of different isolates) is in progress. Acceptable amylose content for PR41326-1 and PR41325B was also observed.

In the development of exerted stigma during F4 generation, 160 plants (consist of sister lines from six breeding population) were selected to have 31-87 % SER. On the other hand, 100% SER was observed from three sister lines in F3 generation. The identification of new maintainer lines having two Xa genes ensures that this may provide resistance to BLB. The acceptable grain quality of developed maintainer lines guarantees to improve grain qualities of succeeding generation of hybrids. The identification of lines having high stigma exertion rate also indicates the possibility of increasing the seed yield of A X R F1 in the future.

**High Yielding Environment Adaptability Test and F1 Seed Production** *JV Galapon, MAU Tabil, and ML Pini* 

The study evaluated the performance of elite three-line hybrid rice genotypes and assessed reproducibility of its corresponding F1 seeds in high yielding environment of Region 02.

Based on the demonstration of the public hybrids across locations, Mestiso 20 recorded the highest yield of 10.02 t/ha. Mestizo 1 yielded 6.65 t/ha while Mestiso 73 registered 9.12 t/ha. The experiment used the recommended rate of fertilizers needed per hectare: 120-60-60 during DS and 90-60-60 during WS with four splits of application, complete fertilizer (14-14-14) for basal (7-10 days after transplanting-DAT), combination of complete and urea (46-0-0) for the second application (30 DAT), urea (46-0-0) for the third application (37 DAT), and Muriate of potash (0-0-60) during 50% heading.

In the adaptability test of the promising hybrids/lines, nine entries were screened and evaluated. The yield data ranged from 4.92t/ha to 6.87t/ha. PR47780H out-yielded the other test entries with 6.87 t/ha yield against the check variety Mestiso 55 with only 5.03 t/ha yield. Likewise, eight promising lines have higher yield than Mestiso 55, which has a yield advantage of 5.17-36%.

In F1 seed production, the performances of the parental were closely monitored during flowering period for synchronization. Differential seeding of the parental of Mestiso 55 was evaluated for both DS and WS under Isabela condition. In DS 2017, for Aline seed production (AXB), two sets of experiment were established with a differential seeding of 1-4-7 (Aline-B1-B2), and 1-1-7 (Aline-B1-B2). Results showed that 1-4-7 (Aline-B1-B2) was the ideal differential seeding for AXB seed production, which produced 8 kg seed yield as compared with 1-1-7(Aline-B1-B2), which yield nothing. In WS 2017, differential seeding of 1-3-7 (Aline-B1-B2) exhibited higher seed yield (4.7 kg) than 1-1-4 (Aline-B1-B2), which did not produce any yield because the Aline and B line did not synchronize.

In AXR seed production, the ideal differential seeding was 1-5-7 (R1-R2-Aline) during DS 2017, which resulted in a higher seed yield (192 kg) than 1-5-5 or R1-R2-Aline (96kg). In WS 2017, given a limited area for the experiment, 1-3-10 (R1-R2-Aline) was the ideal differential seeding for the F1 seed production with 3 kg seed yield. 1-5-8 (R1-R2-Aline) registered no yield.

# Identification and Use of Wide Compatibility Genes (S5n) for Enhancing Heterosis in Rice

IG Pacada, CC Ringor, MTF Celestino, and TMM Pascual

Wide compatibility varieties (WCVs) are special class of rice germplasm that is able to produce fertile hybrids when crossed to other rice subspecies like indica x japonica; indica x javanica; japonica x javanica. It contains wide compatibility gene (WCG) or neutral allele (S5n) that resolves incompatibility or fertility barrier demonstrated in crossing two dissimilar subspecies or cultivar group.

The use of molecular marker speeds up the process of mining rice germplasm with S5n allele, thus this study. Using S5-MMS marker, no S5n allele was detected in 10 hybrid parent lines (maintainer and restorer of released hybrids). However, one exhibited positive for S5n allele among the six inbred line screened. This potential WCV exhibited >70% fertility when crossed to both Indica and Japonica tester. Thirteen out of 49 Javanica germplasm exhibited positive for S5n allele and conventional confirmation is in progress. Among 49 Javanica germplasm evaluated for the presence of S5n allele, 13 exhibited positive for S5n allele.

The mining of WCV among Philippine germplasm provides significant information for both hybrid and inbred rice breeders. The identification of wide compatibility gene is considered as a "game changer" in the field of rice breeding research. This may facilitate to resolve the fertility barrier; thus, widen the gene pool for diversification and the possibility of transferring novel gene from other cultivar (e.g., from japonica, javanica, and bulu group to modern varieties) without incompatibility concerns.

## II. Development of Thermo-sensitive Genetic Male Sterile (TGMS)- based Two-ine Hybrid Rice

Mel Anthony T. Talavera

The project is composed of six interrelated studies. Three studies were devoted for line development of both female and male parents while the remaining three were focused on the generation and evaluation of new, promising, and elite experimental F1 hybrids. Only the few best performing hybrids will be nominated to NCT for countrywide performance evaluation (Figure 1).



Figure 1. Methodological framework of six studies under the TGMS hybrid breeding project

Line development for both female parent (TGMS line) and male parent (pollen parent) involved the utilization of hybridization method and recurrent selection. Segregating generations were handled using the pedigree system. Fixed lines were evaluated for agronomic, yield, flowering habit, and F1 seed reproducibility before used as parents in developing hybrids. For generation of experimental hybrids, the project used manual hand crossing and pollination for new experimental hybrids and isolation free method for generating promising and elite hybrids. For the evaluation of hybrids, the project used three levels of performance trials (HON, HPYT, and AYT). For female development, the project evaluated 206 F2 populations and 1,717 lines coming from F3 to F6 generations at Male Sterile Environment (MSE) while 2,461 plants were shuttled at Male Fertile Environment (MFE) for further evaluation and seed increase. Five new TGMS lines were at final stage of evaluation. These new TGMS lines were used as parents in the development of new experimental hybrids for assessment of combining ability and yielding ability of F1. Moreover, 16 F6 lines were at final phase of evaluation at MFE. Twenty new stable TGMS lines were targeted to produce by 2022.

For pollen parent development, the project evaluated 1,095 lines from F3 to F6 generations. The project's 52new pollen parent were further evaluated in the Pollen Parent Observational Nursery (PPON) for yield, agronomic, and flowering habits. Results showed that seven potential pollen parents were identified based on combination of agronomic, yield, disease and insect resistance, and grain quality. These lines will be further evaluated in the Pollen Parent Yield Trial (PPYT) in WS 2017. For PPYT established during DS 2017, one entry out-yielded the inbred check by 2.93%. Promising pollen parent identified in the PPYT will be used as male parent in developing new two-line experimental hybrids. For WS 2017 trial, data gathering, and harvesting is still ongoing.

There were 963 new experimental hybrids developed and was evaluated in Hybrid Observational Nursery (HON). Of the 400 new experimental hybrids evaluated during the WS trial, 13 hybrids were identified superior than both inbred and hybrid checks. Highest yielding entry during the season was 10,381 kg/ha. All the promising hybrids identified had passed the grain evaluation for chalkiness.

In DS 2017, 24 promising hybrids were evaluated. HPYT 602 performed better than the best hybrid check, PRUP 10. It produced yield of 6,758 kg/ha, which was higher than PRUP 10 by 2.47%. This hybrid had heading date same as the popular hybrid Mestiso 19 with 90 DAS. It was 111 cm tall and produced 10 tillers in a hill. In terms of grain quality, it passed chalkiness evaluation and had intermediate AC and GT.

During DS 2017, 13 hybrids were further evaluated in replicated trial. Result showed that no hybrid entries yielded higher than the hybrid checks Mestiso 20 and PRUP 10. Highest yielding experimental hybrid, Advance Yield Trial (AYT) 168 recorded grain yield of 6,339 kg/ha followed by AYT 171 with 6,242 kg/ha and AYT 169, 6,228 kg/ha. These three hybrids were statistically same with the hybrid checks Mestiso 20 and PRUP 10.

A promising hybrid PRUP 13 is now in the final stage of development before it will be nominated to NCT for evaluation. Basic seed production protocol for this hybrid in the pipeline was conducted during the

year. For days towards heading, the female parent used was 6 days earlier compared with the pollen parent. On the other hand, P line was 20 cm taller on the average compared with the new S line. This height advantage is considered ideal for outcrossing. Optimization of this protocol will commence during DS 2018.

# Development of New and Diverse TGMS Lines through Hybridization and Selection

MAT Talavera, MLG Ortiguero, and EE Sajise

This study aimed to develop new and diverse TGMS lines through hybridization and selection. Hybridization method was used to transfer TGMS trait into individuals of improved genetic background. Segregating generations were handled using pedigree system while evaluation of breeding materials and fixed lines was done using shuttle breeding method. Sterility of TGMS breeding materials were evaluated at Male Sterile Environment (MSE) while fertility and seed generation were assessed at Male Fertile Environment (MFE).

Sixty-eight F2 populations and 1,024 lines from F3 to F6 were evaluated at MSE while 1,076 ratooned plants were brought to MFE for further evaluation and seed increase. Sixteen F6 lines were at final phase of evaluation at MFE. These fixed lines will be further evaluated before using as female parent in developing new two-line hybrids.

# Development of New and Diverse TGMS Lines through Recurrent Selection

Mona Liza G. Ortiguero and BT Salazar

Using recurrent selection method, this study aimed to develop more diverse and improved TGMS lines to be used as female parents in generating two-line hybrids. Recurrent selection, which is a repeated cycle of intercrossing, recombination, and selection, concentrates on desirable traits of fewer individuals in the population. Composite intercrossing populations were established at MSE as TGMS-based system was employed. Seeds from selected desirable sterile and fertile plants from the composite populations will be used for the next intercrossing and selection cycle. Segregating lines extracted from the composite populations were handled using the pedigree system and shuttling of breeding materials between MSE and MFE was followed.

Two composite populations were established for 2017. Five lines were observed to be completely sterile with good phenotypic acceptability at MSE, plant height ranging from 78 to 88 cm, and tillers from 10 to 15.

These lines were used as parents in the development of experimental hybrids for preliminary testing of combining ability and yielding ability of F1 in WS 2017.

# Identification and Development of Pollen Parents for Two-line Hybrids through Recurrent Selection

Mel Anthony T. Talavera and Mona Liza G. Ortiguero

Availability and identification of potentially good-performing pollen parents are essentials in hybrid development programs for both twoline and three-line system. This study aimed to develop pollen parents with good pollen-shedding ability and are high yielding, resistant to pests and diseases, and with good grain quality. The study employed recurrent selection, which is a cyclical improvement technique aimed at gradually concentrating desirable alleles in a population. Inter-breeding populations were also established using the genetic male sterility mechanism. Extraction of segregating lines in developing new pollen parents were handled by the pedigree method of breeding. The recurrent intercrossing and selection were continued.

Three composite populations were established for the year. There were 1,095 lines from F3 to F6 generations established and evaluated while 52 and 28 entries comprised the Pollen Parent Observation Nursery (PPON) and Pollen Parent Yield Trial (PPYT). In PPON, seven pollen parents out-yielded the check variety used (NSIC Rc222). For PPYT, one entry surpassed the yield of checks PSB Rc82 and NSIC Rc222 by 18.86 and 2.93%, respectively.

### **Development of Two-line Experimental Hybrids**

Mona Liza G. Ortiguero and Babylyn T. Salazar

To increase the chances of finding good heterotic hybrids, there is a need to increase the number of experimental hybrids for evaluation and testing. Promising TGMS lines and pollen parents were assembled in a test cross nursery to generate two-line experimental hybrids. Seeds of these experimental hybrids were produced either through hand crossing or using isolation-free method. Hand crossing involved clipping of florets of TGMS parent, pairing with corresponding pollen parents, covering the panicles of TGMS and pollen parents with cellophane bag, and tapping the bag during pollination. Isolation-free method, on the other hand, involved planting selected pollen parents in plots with four border rows and four vacant spaces in between single rows of pollen parent in the middle of the plot, staggered planting of TGMS parent, and lifting and planting of TGMS parents in the vacant spaces during onset of flowering followed by supplementary pollination.

For 2017, 50 TGMS lines and 284 pollen parents were assembled to constitute the test cross nursery. Nine hundred sixty-three new experimental hybrids were generated.

### **Evaluation and Field Performance Testing of Promising Two-line Hybrids** *MAT Talavera, MLG Ortiguero, and BT Salazar*

To find heterotic two-line hybrids, elaborate testing of performance of new and promising hybrids are needed to determine the most promising lines in terms of yield heterosis per se and other characteristics. The study evaluated the performance of new and promising experimental hybrids for yield, pest resistance, and grain quality. Three levels of yield trials were used in this study. In HON, 13 promising hybrids were identified superior than the check varieties used. Highest yielding experimental hybrid recorded 10,381 kg/ha. For HPYT, one hybrid was identified higher yielding than the check with mean yield of 6,758 kg/ha. For AYT, no hybrid surpassed the yield of hybrid check PRUP 10; however, three hybrids had mean yield statistically same with the hybrid check.

### F1 Seed Production of Two-line Hybrids for Testing and Evaluation

Babylyn T. Salazar and Mel Anthony T. Talavera

A successful hybrid rice breeding program requires a system for mass producing adequate number of experimental hybrids and corresponding quantity of hybrid seeds for various stages of testing and evaluation. This study aimed to produce sufficient amount of F1 seeds for testing and evaluation and to initially determine the F1 seed production capacity of promising hybrids. To eliminate inferior hybrids, the experimental hybrids undergo evaluation under unreplicated trials. Subsequently, hybrids were evaluated in replicated trials and larger plot sizes. Isolation-free method by Virmani et. al. was adopted in producing seeds for HPYT and AYT while smaller SxP seed production plots were established for hybrids intended for NCT, agronomic trials, on farm trials, and field demonstration plots.

Two hundred twenty-four experimental hybrids were reconstructed for PYT and AYT. Adequate amount of seeds of purple based M19 and M20 were also produced. Both hybrids will be forwarded to NCT under the essentially-derived category. Initial data of the new hybrid in the pipeline showed that the P line possesses the necessary height advantage over the S line, which is ideal for a successful outcrossing. However, there is still the need to optimize the appropriate seeding interval of the parentals in the succeeding seasons.

### **III. Hybrid Seed and Seed Production Research** Susan R. Brena

A strong seed technology program is necessary for the implementation of hybrid seed production and distribution, which will serve as a bridge between research and agriculture along which varieties and seeds travel. The project covers all aspects of seed production from basic seed research, seed multiplication, harvesting, post-harvest processing, storage, and seed quality control. This project was implemented with the following objectives: 1) conduct seed production trials of TGMS hybrids in different locations; 2) establish flowering behavior and seed production capacity of parental lines; 3) find suitable alternative in the control plot in TGMS seed certification; 4) develop simple procedure for pollen collection and storage; 5) purify parental lines of M32 and M55 and characterize component lines based on agro-morphological characteristics; 6) produce nucleus and breeder seeds of newly released public PhilRice – bred hybrids; 7) improve seed yield of newly released public hybrids through appropriate row ratio and GA3 concentration; and 8) evaluate the potential of mid-elevation storage environment for hybrid parental lines.

Eight studies were implemented: 1) Identification of best location and time of the year/season optimum for seed production and quality; 2) Flowering behavior and seed production capacity of parental lines in different locations and seasons; 3) Development of possible alternative for the control plot in TGMS hybrid seed certification; 4) Strategies for pollen harvest and storage; 5) Nucleus and breeder seed production studies for new recommended hybrid varieties; 6) Hybrid nucleus and breeder seed production; 7) Optimizing row ratio and GA3 concentration to increase seed yield of newly released public hybrids (CES, Negros, Midsayap); and 8) Effectiveness of storing seeds of hybrid parental lines at mid-elevation sites under ambient conditions.

TGMS lines of PRUPTG102 seed production was integrated in the parental seed production in Don Salvador Benedicto, Negros Occidental. This was a long-term site for producing seed of TGMS lines of NSIC Rc202H and NSIC Rc204H. Three MFE sites were evaluated for seed production capacity of the TGMS lines planted at different times.

Based on Grow Out Test (GOT), genetic purity of the seed lots produced was higher than the standard 97% that is acceptable to hybrid seed growers. However, genetic purity assessment using SSR molecular markers, RM1, RM126, and RM511 detected impurities in one seed lot, DSB O1 resulting in only 92% genetic purity. Two seed lots had 100% genetic purity after DNA analysis.

S x P seed production of Mestiso 19 was tried in DS and WS in PhilRice-CES and PhilRice-Negros. The trial was conducted to identify new site for producing TGMS seed of this variety. Many hybrid seed growers in existing TGMS seed production areas do not seed produce Mestiso 19 owing to its low seed yield. The trial in CES used 3:10 row ratio while the trial in PhilRice-Negros tested several row ratios: 3:6; 3:8; 3:10, and 3:12. SxP seed production in both sites was delayed owing to time of isolation. Fertilizer application was done following the general recommendation. However, gibberellic acid (GA3) spray was applied at 250 g/ha at CES and 100 g/ha at PhilRice-Negros. Application was done in splits in both locations. At CES, GA3 was initially sprayed at 10-15% heading and second application was done 30-45% heading. At PhilRice-Negros, 60% GA3 was sprayed at 20-30% heading and 40% was sprayed at 40-60% heading or two days after the first application. In both sites, supplementary application using rope started at 9 am; from beginning anthesis until P-lines ceased to flower. Percent seed yield and seed yield per S-line row in PhilRice-Negros were monitored.

Owing to delayed SxP establishment in both sites, seed yield was very low. In DS, 74 kg/ha seed yield was attained in PhilRice-CES. There was good blooming in the TG101M but PRUPTG101 (S-lines) was severely affected by stem borer, known as white heads. At CES, the cold spell experienced in January and early February delayed planting further until the end of February. Time isolation is a key strategy in SxP production even in WS but the disease occurrence poses challenge on seed yield.

At PhilRice-Negros, DS trial proved possible but delayed establishment resulted in low seed yield. Among the row ratios tried, 3:6 appeared to be better in terms of seed yield than the others, though, seed yield difference was not significant. It was shown that S-lines rows near the P-line rows exhibited higher seed yield than rows farther from the P-lines.

TGMS seed production is governed by the latest Administrative Order No. 8 series 2012, which requires 40 m2 control plot (CP). One CP can be used by hybrid seed growers provided they established the SxP seed production of one TGMS variety on the same day. The CP is established to measure the degree of selfing in the SxP. However, hybrid seed growers find it difficult to establish the CP owing to isolation issues. More hybrid seed growers will engage in TGMS seed production if there will be a substitute for the CP. This study intended to find an alternative to establishing CP by bagging certain number of S panicles in the SxP plot. This is anchored on the idea that these bagged S panicles should have unfilled grains if not pollinated by the P-lines; otherwise, they have self-pollinated. At CES, around 500 panicles were bagged in SxP plot planted using 3:10 row ratio. In the hybrid seed growers' field, 1,500 S-lines panicles were bagged in three SxP fields owned by three hybrid seed growers using 2:12 row ratio. Five hundred panicles were bagged per SxP seed production field. Each field served as a replicate. At PhilRice-Negros, 1,200 panicles were bagged at 400 panicles per SxP field. Each field served as a replicate.

Bagging of S-line panicles in SxP seed production before panicle emerged was tried at CES in DS, hybrid seed grower fields in Banay-banay, Davao Oriental, and PhilRice-Negros in WS. In DS trial, 0.32 gm filled grains were collected in the bagged panicles and around 0.51 gm in the CP. In CES, seed yield in SxP plot was only 74 kg/ha owing to high incidence of disease in the S-lines. Result of bagging S-line panicles in Banay-banay, Davao Oriental was quite similar with the result obtained in CES in DS. Filled grains in the CP was 26.3 g while only 9.53 g filled grains were obtained in the bagged panicles. Weight of the filled grains obtained in the bagged S-line panicles was lower than the weight of the filled grains in CP; making them good candidates to replace the CP. However, general conclusion is yet be drawn pending on the availability of data on weight of the seed yield in the three hybrid seed growers' SxP seed production plot. The row ratio and the seed yield in SxP is considered in computing the maximum allowable weight of filled grains in the CP.

Attainment of high seed yield in hybrid seed production is a function of the amount of pollen that the male parent can shed during pollination. Low pollen load of male parent (TG101) of Mestiso 19 leads to low seed yield. Artificial pollination, through collection and storage of sufficient amount of pollen may provide a solution to the problem. Artificially loading additional pollen onto stigmas of female parent of Mestiso 19 (PRUPTG101) may increase seed yield. The study was conducted during wet and dry season of 2016-2017 at PhilRice CES to develop technique in pollen collection and storage of TG101M. Pollens were collected at anthesis, 30 minutes and 1 hour after anthesis. The pollens are stored in amber glass, paper and zip lock plastics; then kept in different 24, 48 and 72 hours in different temperatures (280C, 50C, -50C). Pollens were grown in three media [media 1 (10%PEG+20%sucrose+0.05%CaCl2+0.01%Boric acid); media 2 (3% Ca(NO3)2+5%Sucrose+1% Agarose+10 % Boric acid); and media 3 (4% Calcium nitrate+14% Sucrose+1% Potassium Nitrate+1% Boric acid)] and were evaluated under microscope for pollen tube growth. Pollen viability decreased after anthesis collection. Growing grains in media 2 preserved pollen viability even 1 hour after anthesis of pollen collection. Pollen germination decreased over time regardless of media used. Pollen tube length was consistently high at anthesis. The length of pollen tube growth from medium 2 differed between 30 min and 1 hour. More than 50% viable pollen was achieved after 24 hours of storage. Pollen viability and germination decreased beyond 24 hours. To increase the viability of collected pollen for possible artificial pollination, grains must be collected at anthesis and stored immediately in amber glass under cold storage at negative 5°C.

Activities of the study focused on purification, production, and distribution of basic seeds of Mestizo 1 (PSB Rc72H) and Mestiso 20 (NSIC Rc204H) parent lines. The purification process involved A-line evaluation and paired-cross generation for three-line hybrids and plant selection at MFE and evaluation at MSE for TGMS-based hybrids. Nucleus seed production activities for Mestizo 1 were done during dry season. There were 2318 A-line plants evaluated to generate 474 effective IR58025AXB paired-crosses. The crosses will be evaluated in 2018 to produce nucleus seed. During DS, the amount of nucleus seed produced is enough to plant 6 ha breeder seed production plot. The materials were stored as future source of planting material for breeder seed production. In the case of Mestiso 20, purification was initiated by selecting 1,000 true-to-type PRUPTG102 plants during WS. The materials will be evaluated in 2018 DS. Breeder seeds of the component lines of Mestizo 1 (A, B, R-line) and Mestiso 20 (S, P-line) were produced in both seasons at Los Baños and Benguet. More than 1 ton-female parents were produced within the requirement of the commercialization program. Breeder seeds of the male parents of the commercial hybrids were also produced in sufficient amount. The average yield for AxB seed production was 600 kg/ha (500-700 kg/ha). In the WS, the harvested amount of PRUPTG102 can plant approximately 25 ha foundation seed production of S-line. The group also produced F1 hybrids of Mestiso 20 during the dry season for research purposes. Breeder seeds of the parent lines of Mestiso 20 and Mestizo 1 were dispatched to PhilRice stations in Isabela, Negros, and Midsayap for foundation seed production. Sufficient volume of breeder seeds of public hybrid parents are kept in the cold rooms at Seed Processing and Storage Facility at Los Baños. They were distributed to PhilRice stations and accredited hybrid seed growers on request.

Purification and multiplication of the parent lines and F1 of released hybrids Mestiso 32 (NSIC Rc250H) and Mestiso 73 (NSIC Rc446H) were conducted. Seed purification activities for essentially derived TGMS-hybrids PRUP 11 and PRUP 12 were also started this year. Pair-crossing activities for IR68897A X B (Mestiso 32) were done. Three hundred seventy-one (32%) entries were found to be completely sterile (CS) and lifted for crossing with corresponding B-lines. The paired-crosses were processed and will be evaluated for sterility, uniformity, trueness, and other agro-morphological parameters in 2018 DS. Restorer line (IR73013R) was also produced during the season. Sufficient amount of S-line (PRUP TG101) and P-line (SN 758) are already available and activities were mainly focused on F1 seed production for distribution to selected sites for further evaluation together with the produced F1 hybrids of Mestiso 32 produced in the wet season. Nucleus and breeder seeds of the purple-version of the pollen parents of Mestiso 19 (TG101M-P) and Mestiso 20 (TG102M-P) developed by the TGMS group at Los Baños were multiplied for further research. Packaging of seed production and familiarity kits for Mestiso 32 is currently on-going. Mestiso 73 seed production kits were packaged and distributed to seed

cooperators in Davao Oriental and PhilRice Stations in Isabela, Negros, Midsayap and Nueva Ecija. The group at Los Baños with assistance from TGMS Breeding researchers established a seed production protocol for this hybrid now being used as basis for further studies and optimization researches.

One of the studies under the project aimed to improve seed yield of M73 (NSIC Rc446H), a PhilRice-bred hybrid released in 2016. A 4x3 factorial experiment was laid out in strip-plot design with GA3 concentration (0, 100, 150, and 200 g/ha) and row ratio or ratio of male to female parentals (2:6, 3:8, and 3:10) as treatments. Preliminary result of the study showed that differences in seed yield were attributed to the separate effects of row ratio and GA3 concentration. Across treatments, seed yield ranged from 0.7 to 1.5 t/ha. Plants harvested in plots under 2:6 or 3:8 row ratio exhibited 15% more seed yield compared to those harvested from 3:10 plots. Generally, applying higher concentration of GA3 resulted in significantly taller plants and produces higher see yield. Another set-up is proposed to be conducted DS 2018 to verify the results as GA3 was not properly applied owing to frequent rainfall during the flowering stage of the experimental plants.

The quality of hybrid parentals stored in mid-elevation environment was evaluated in this study. A 3x2x2x2 factorial experiment was laid in split-split-split plot with storage environment (cold room, mid elevation, ambient), storage container (hermetic sack, ordinary sack), seed quantity (1 sack, ½ sack), and parental (S and P) as treatments with three replications. Sampling for seed germination and seedling vigor is done every two weeks for six months. Regular monitoring of seed and air temperature and relative humidity is done. Based on initial results, seeds stored in ordinary sack under ambient conditions exhibited the lowest germination rate (83%) regardless of seed quantity, and the type of parental stored, after 10 weeks of storage. Seeds packed in hermetic sack under cold room or middle elevation environment, on the other hand, had comparable germination rates (96%). Based on preliminary results, parental seeds are best packed in hermetic sack and stored under cold room or middle elevation environment.

## Identification of Best Location and Time of the Year/Season Optimum for Seed Production and Quality

SR Brena, AGF (PhilRice CES); and M Osano-Palanog (PhilRice Negros)

SxP seed production of Mestiso 19 was tried in DS and WS at PhilRice-CES and PhilRice-Negros. The trial was done to identify new site for TGMS seed production other than Davao Oriental. The trial in CES used 3:10 row ratio while the trial in PhilRice-Negros used 3:10 and the other row ratios, 3:6; 3:8; and 3:12. SxP seed production in both sites was delayed

owing to time of isolation. Fertilizer application was done following the general recommendation. However, GA3 spray was applied at 250 g/ha in CES and 100 g/ha in PhilRice-Negros. Application was done in splits in both locations. At CES, GA3 was initially sprayed at 10-15% heading and second application was done 30-45% heading. At PhilRice-Negros, 60% GA3 was sprayed at 20-30% heading and 40% was sprayed at 40-60% heading or two days after the first application. In both sites, supplementary application using rope started at 9 am; from beginning anthesis until P-lines ceased to flower. Percent seed yield and seed yield per S-line row in PhilRice-Negros were monitored.

Owing to delayed SxP establishment in both sites, seed yield was very low. In DS, CES registered 74 hg/ha seed yield. There was good blooming in the TG101M but PRUTG101 (S-lines) was severely diseased. The cold spell experienced in January and early February also extended planting until the end of February. Time isolation was favored even in WS but disease became the most disastrous problem.

At PhilRice-Negros, DS trial proved possible but delayed establishment resulted in low seed yield. Among the row ratios tried, 3:6 appeared better than other row ratios tried, though, seed yield difference was not significant. It was shown that S-lines rows very near the P-line rows exhibited higher seed yield than rows farther from the P-lines. To convince hybrid seed growers to re-engage in seed producing Mestiso 19, recommendations from this study may be replicated their seed production field.

## Flowering Behavior and Seed Production Capacity of Parental Lines in Different Locations and Seasons

SR Brena, AG Ferriol (PhilRice CES); and M Osano-Palanog (PhilRice Negros)

TGMS lines of PRUPTG102 seed production was integrated in the parental seed production in Don Salvador Benedicto, Negros Occidental. Three MFE (male fertile environment) sites were evaluated in the study for seed production capacity of the TGMS lines planted at different times. The first MFE site, which was planted the earliest, gave only 510 kg/ha seed yield and was considered the lowest. The low seed yield was due to bird damage. Spikelet fertility and percent seed were high but seed yield was low owing to high percentage shedding of grains during bird movement. MFE site 2 gave the highest yield at 1,363 kg/ha while MFE site 3 had only 923 kg/ha seed yield. The highest seed yield in MFE site 2 was greatly enhanced by the good irrigation system in the area. The average seed yield obtained in the 3 MFE sites was 932 kg/ha.

Genetic purity of the seed lots produced after GOT was higher than the standard 97%. However, genetic purity assessment using SSR molecular markers, RM1, RM126, and RM511 detected impurities in seed lot DSB O1, which resulted in only 92% genetic purity. However, 2 seed lots had 100% genetic purity after DNA analysis.

# Development of Possible Alternative for the Control Plot in TGMS Hybrid Seed Certification

SR Brena, AG Ferriol (PhilRice CES); and M Osano-Palanog (PhilRice Negros)

TGMS seed production is governed by the latest Administrative Order No. 8 series 2012, which requires 40 m2 control plot (CP). One CP can be used by hybrid seed growers provided they established the SxP seed production of one TGMS variety on the same day. The CP is established to measure the degree of selfing in the SxP. However, hybrid seed growers find it difficult to establish the CP owing to isolation issues. More hybrid seed growers will engage in TGMS seed production if there will be a substitute for the CP. This study was conducted to look for possible alternative for the CP. At CES, around 500 panicles were bagged in SxP plot planted using 3:10 row ratio. In the hybrid seed growers field, 1,500 S-lines panicles were bagged in three SxP fields owned by three hybrid seed growers using 2:12 row ratio. Five hundred panicles were bagged per SxP seed production field. Each field served as a replicate. At PhilRice-Negros, 1,200 panicles were bagged at 400 panicles per SxP field. Each field served as a replicate.

Bagging of S-line panicles in SxP seed production before panicle emerged was tried at CES in DS, hybrid seed grower fields in Banaybanay, Davao Oriental and PhilRice-Negros in WS. In DS trial, 0.32 g filled grains were collected in the bagged panicles and around 0.51 g in the CP. At CES, seed yield in SxP plot was only 74 k/ha owing to high incidence of disease in the S-lines. Result of bagging S-line panicles in Banaybanay, Davao Oriental was quite similar with the result obtained in CES in DS. Filled grains in the CP was 26.3 g while only 9.53 g filled grains were obtained in the bagged panicles. Weight of the filled grains obtained in the bagged S-line panicles was lower than the weight of the filled grains in CP; making them good candidates to replace the CP.

### Strategies for Pollen Harvest and Storage in View of Increasing Pollination and Yield in Hybrid Seed Production of Mestiso 19

AG Ferriol, SR. Brena, and ML Marturillas

Attainment of high seed yield in hybrid seed production is a function of the amount of pollen that the male parent can shed during pollination. Low pollen load of male parent (TG101) of Mestiso 19 results in low seed yield. Artificial pollination, through collection and storage of sufficient amount of pollen may provide a solution to the problem. Additional pollen

can be artificially loaded onto stigmas of female parent of Mestiso 19 (PRUPTG101), which may increase seed yield. The study was conducted during wet and dry season 2016-2017 at PhilRice CES to develop technique in pollen collection and storage of TG101M. Pollens were collected at anthesis, 30 min and 1 h after anthesis, then stored in amber glass, paper, and ziplock plastics. They were kept in 24, 48 and 72 hours at different temperatures (280C, 50C, -50C). Pollens were grown in three media [media 1 (10% PEG+20% sucrose+0.05% CaCl2+0.01% Boric acid), media 2 (3% Ca(NO3)2+5% Sucrose+1% Agarose+10 % Boric acid), media 3 (4% Calcium nitrate+14% Sucrose+1% Potassium Nitrate+1% Boric acid)] and were evaluated under microscope for pollen tube growth. Pollen viability decreased after anthesis collection. Growing grains in media 2 preserved pollen viability even after 1 hour after anthesis of pollen collection. Pollen germination decreased over time regardless of media used. Pollen tube length was consistently high at anthesis. The length of pollen tube growth from medium 2 differed between 30 min and 1 h. More than 50% viable pollen was achieved after 24 h of storage. Pollen viability and germination decreased beyond 24 h. To increase the viability of collected pollen for possible artificial pollination, grains must be collected at anthesis and stored immediately in amber glass under cold storage at negative 5°C.

### **IV. Hybrid Nucleus and Breeder Seed Production Research** and Maintenance

### Hybrid Nucleus and Breeder Seed Production

LV Guittap, WB Abonitalla, SR Brena, MAT Talavera, and TM Masajo

Activities of the study focused on purification, production, and distribution of basic seeds of Mestizo 1 (PSB Rc72H) and Mestiso 20 (NSIC Rc204H) parent lines. The purification process involved A-line evaluation and paired-cross generation for three-line hybrids and plant selection at MFE and evaluation at MSE for TGMS-based hybrids. Nucleus seed production activities for Mestizo 1 was done during dry season. There were 2,318 A-line plants evaluated to generate 474 effective IR58025AXB pairedcrosses. The crosses will be evaluated in 2018 to produce nucleus seed. The materials were stored as future source of planting material for breeder seed production. In the case of Mestiso 20, purification was initiated by selecting 1,000 true-to-type PRUP TG102 plants during the wet season. The materials will be evaluated in DS 2018. Breeder seeds of the component lines of Mestizo 1 (A, B, R-line) and Mestiso 20 (S, P-line) were produced in both seasons at Los Baños and Benguet. More than 1 ton of female parents were produced during the period, which is required of the commercialization program. Breeder seeds of the male parents of the commercial hybrids were also produced in sufficient amount. The average yield for AXB seed

production in 2017 was 600 kg/ha (500-700 kg/ha). In the wet season, the harvested amount of PRUP TG102 can plant approximately 25 ha foundation seed production of S-line. The group also produced F1 hybrids of Mestiso 20 during the dry season for research. Breeder seeds of the parent lines of Mestiso 20 and Mestizo 1 were dispatched to PhilRice stations in Isabela, Negros, and Midsayap for foundation seed production. Sufficient volume of breeder seeds of public hybrid parents are kept in the cold rooms at Seed Processing and Storage Facility at Los Baños. They are distributed to PhilRice stations and accredited hybrid seed growers on request.

### Nucleus and Breeder Seed Production Studies for New Recommended Hybrid Varieties

LV Guittap, WB Abonitalla, SR Brena, MAT Talavera, and TM Masajo

Purification and multiplication of the parent lines and F1 of released hybrids Mestiso 32 (NSIC Rc250H) and Mestiso 73 (NSIC Rc446H) was conducted. Pair-crossing activities for IR68897A X B (Mestiso 32) was done. Three hundred seventy-one (32%) entries were found to be completely sterile (CS) and lifted for crossing with corresponding B-lines. The pairedcrosses were processed and will be evaluated for sterility, uniformity, trueness and other agro-morphological parameters in the 2018 dry season. Restorer line (IR73013R) was also produced during the season. Sufficient amount of S (PRUP TG101) and P-line (SN 758) are already available and activities mainly focused on F1 seed production for distribution to selected sites for further evaluation together with the F1 hybrids of Mestiso 32 produced in 2017 WS. Nucleus and breeder seeds of the purple-version of the pollen parents of Mestiso 19 (TG101M-P) and Mestiso 20 (TG102M-P) developed by the TGMS group in Los Baños were multiplied for further research. Packaging of seed production and familiarity kits for Mestiso 32 is currently on-going. Mestiso 73 seed production kits were packaged and distributed to seed cooperators in Davao Oriental and PhilRice Stations in Isabela, Negros, Midsayap, and Nueva Ecija.

### **Optimizing Row Ratio and GA3 Concentration to Increase Seed Yield of Newly-released PhilRice-bred Hybrids** Babylyn T. Salazar

Release of a new variety should be accompanied with approporiate management practices necessary for increased seed yield. Synchronized flowering of the parentals leads to increased seed yield in hybrid. According to Chinese hybrid rice experts, achievement of optimum seeding interval, row ratio, and GA3 timing and application are among the methods to achieve ideal flowering synchronization of parentals. The study aimed to improve seed yield of M73 (NSIC Rc446H), a PhilRice-bred hybrid released

in 2016. A 4x3 factorial experiment was laid out in strip-plot design with GA3 concentration (0, 100, 150, and 200 g/ha) and row ratio or ratio of male to female parentals (2:6, 3:8, and 3:10) as treatments. Preliminary result of the study showed that differences in seed yield were attributed to the separate effects of row ratio and GA3 concentration. Across treatments, seed yield ranged from 0.7 to 1.5 t/ha. Plants harvested in plots under 2:6 or 3:8 row ratio exhibited 15% more seed yield compared to those harvested from 3:10 plots. Generally, applying higher concentration of GA3 resulted in significantly taller plants, which also produce higher seed yield. Another set-up is proposed to be conducted DS 2018 to verify the results because GA3 was not properly applied due frequent rainfall during the flowering stage.

## Effectiveness of Storing Seeds of Hybrid Parentals at Mid-elevation Sites under Ambient Conditions

BT Salazar, W Abonitalla, and LV Guittap

The challenge for PhilRice is to ensure that the quality of its seeds is maintained as it reaches the clients. Since TGMS hybrid commercialization was launched, the demand for the seeds increased along with the volume of seed produced. This translates to the need of efficient storage system as the seeds are of high value (P750/k). TGMS lines or S lines are currently planted in mid- elevation areas of Nueva Vizcaya, Laguna, Quezon, and Negros Occidental, but stored in seed warehouses at PhilRice CES, Isabela, Los Baños, and Negros. The hypothesis is that the natural cool weather in mid-elevation environment where these seeds are produced can contribute to the maintenance of seed quality. The study aimed to evaluate the quality of hybrid parentals stored in mid-elevation environment. A 3x2x2x2 factorial experiment was laid in split-split-split plot with storage environment (cold room, mid elevation, ambient), storage container (hermetic sack, ordinary sack), seed quantity (1 sack,  $\frac{1}{2}$  sack), and parental (S and P) as treatments with three replications. Sampling for seed germination and seedling vigor is done every 2 weeks for 6 months. Regular monitoring of seed and air temperature and relative humidity is done. Based on initial results, seeds stored in ordinary sack under ambient conditions exhibited the lowest germination rate (83%) regardless of seed quantity, and the type of parental stored, after 10 weeks of storage. Seeds packed in hermetic sack under cold room or middle elevation environment, on the other hand, had comparable germination rates (96%). Based on preliminary results, parental seeds are best packed in hermetic sack and stored under cold room or middle elevation environment.

#### Abbreviations and acronymns

ABA – Abscicic acid Ac – anther culture AC – amylose content AESA - Agro-ecosystems Analysis AEW - agricultural extension workers AG – anaerobic germination AIS – Agricultural Information System ANOVA - analysis of variance AON – advance observation nursery AT – agricultural technologist AYT - advanced yield trial BCA – biological control agent BLB – bacterial leaf blight BLS – bacterial leaf streak BPH – brown planthopper Bo - boron BR – brown rice BSWM - Bureau of Soils and Water Management Ca - Calcium CARP - Comprehensive Agrarian Reform Program cav – cavan, usually 50 kg CBFM – community-based forestry management CLSU - Central Luzon State University cm - centimeter CMS – cystoplasmic male sterile CP – protein content CRH - carbonized rice hull CTRHC - continuous-type rice hull carbonizer CT – conventional tillage Cu – copper DA – Department of Agriculture DA-RFU - Department of Agriculture-Regional Field Units DAE – days after emergence DAS – days after seeding DAT – days after transplanting DBMS - database management system DDTK – disease diagnostic tool kit DENR – Department of Environment and Natural Resources DH L- double haploid lines DRR – drought recovery rate DS – dry season DSA - diversity and stress adaptation DSR – direct seeded rice DUST - distinctness, uniformity and stability trial DWSR – direct wet-seeded rice EGS – early generation screening EH – early heading

EMBI - effective microorganism-based inoculant EPI – early panicle initiation ET – early tillering FAO – Food and Agriculture Organization Fe – Iron FFA – free fatty acid FFP – farmer's fertilizer practice FFS – farmers' field school FGD – focus group discussion FI – farmer innovator FSSP - Food Staples Self-sufficiency Plan g – gram GAS – golden apple snail GC – gel consistency GIS – geographic information system GHG – greenhouse gas GLH - green leafhopper GPS – global positioning system GQ - grain quality GUI - graphical user interface GWS - genomwide selection GYT – general yield trial h – hour ha – hectare HIP - high inorganic phosphate HPL – hybrid parental line I - intermediate ICIS – International Crop Information System ICT – information and communication technology IMO - indigenous microorganism IF – inorganic fertilizer INGER - International Network for Genetic Evaluation of Rice IP – insect pest IPDTK - insect pest diagnostic tool kit IPM – Integrated Pest Management IRRI – International Rice Research Institute IVC – in vitro culture IVM – in vitro mutagenesis IWM – integrated weed management JICA – Japan International Cooperation Agency K – potassium kg – kilogram KP – knowledge product KSL – knowledge sharing and learning LCC – leaf color chart LDIS - low-cost drip irrigation system LeD – leaf drying LeR – leaf rolling lpa – low phytic acid LGU – local government unit

LSTD - location specific technology development m – meter MAS – marker-assisted selection MAT – Multi-Adaption Trial MC – moisture content MDDST - modified dry direct seeding technique MET – multi-environment trial MFE - male fertile environment MLM - mixed-effects linear model Mg – magnesium Mn – Manganese MDDST - Modified Dry Direct Seeding Technique MOET – minus one element technique MR - moderately resistant MRT – Mobile Rice TeknoKlinik MSE - male-sterile environment MT – minimum tillage mtha<sup>-1</sup> - metric ton per hectare MYT – multi-location yield trials N – nitrogen NAFC - National Agricultural and Fishery Council NBS - narrow brown spot NCT – National Cooperative Testing NFA – National Food Authority NGO - non-government organization NE – natural enemies NIL – near isogenic line NM – Nutrient Manager NOPT - Nutrient Omission Plot Technique NR – new reagent NSIC – National Seed Industry Council NSQCS - National Seed Quality Control Services OF – organic fertilizer OFT – on-farm trial OM – organic matter ON – observational nursery OPAg - Office of Provincial Agriculturist OpAPA – Open Academy for Philippine Agriculture P – phosphorus PA – phytic acid PCR – Polymerase chain reaction PDW - plant dry weight PF – participating farmer PFS – PalayCheck field school PhilRice – Philippine Rice Research Institute PhilSCAT – Philippine-Sino Center for Agricultural Technology PHilMech – Philippine Center for Postharvest Development and Mechanization PCA – principal component analysis

PI – panicle initiation PN – pedigree nursery PRKB – Pinoy Rice Knowledge Bank PTD – participatory technology development PYT – preliminary yield trial QTL - quantitative trait loci R - resistant RBB – rice black bug RCBD - randomized complete block design RDI - regulated deficit irrigation RF – rainfed RP - resource person RPM – revolution per minute RQCS – Rice Quality Classification Software RS4D - Rice Science for Development RSO – rice sufficiency officer RFL – Rainfed lowland RTV – rice tungro virus RTWG – Rice Technical Working Group S – sulfur SACLOB - Sealed Storage Enclosure for Rice Seeds SALT – Sloping Agricultural Land Technology SB – sheath blight SFR – small farm reservoir SME – small-medium enterprise SMS - short message service SN – source nursery SSNM - site-specific nutrient management SSR – simple sequence repeat STK – soil test kit STR - sequence tandem repeat SV – seedling vigor t – ton TCN – testcross nursery TCP – technical cooperation project TGMS – thermo-sensitive genetic male sterile TN – testcross nurserv TOT – training of trainers TPR – transplanted rice TRV - traditional variety TSS - total soluble solid UEM – ultra-early maturing UPLB – University of the Philippines Los Baños VSU – Visayas State University WBPH – white-backed planthopper WEPP - water erosion prediction project WHC – water holding capacity WHO - World Health Organization WS – wet season WT – weed tolerance YA – yield advantage Zn – zinc ZT – zero tillage



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We are a government corporate entity (Classification E) under the Department of Agriculture. We were created through Executive Order 1061 on 5 November 1985 (as amended) to help develop high-yielding and cost-reducing technologies so farmers can produce enough rice for all Filipinos.

With a "Rice-Secure Philippines" vision, we want the Filipino rice farmers and the Philippine rice industry to be competitive through research for development in our central and seven branch stations, coordinating with a network that comprises 59 agencies strategically located nationwide.

We have the following certifications: ISO 9001:2008 (Quality Management), ISO 14001:2004 (Environmental Management), and OHSAS 18001:2007 (Occupational Health and Safety Assessment Series).

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